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Abstract

It has been postulated that occupational exposure to hydrocarbons may damage the kidney and lead to glomerulonephritis and chronic renal failure. As laminin is a ubiquitous basement membrane component that seems to play a central part in the structure and function of basement membranes and as the normal renal filtration process is highly dependent on an intact glomerular basement membrane, the serum laminin concentration was examined in a population of workers exposed to hydrocarbons. The hydrocarbon exposure was assessed by exposure surrogates (exposure duration and exposure score). An interaction between occupational exposure to hydrocarbons and hypertension increased the laminin concentration whereas the laminin concentration decreased in workers exposed for a long time probably because of a selection effect. In a subgroup of printers exposed to toluene whose hippuric acid excretion had been recorded for several years this interaction was confirmed when the hippuric acid excretion was substituted for the other exposure indices. In the exposed group, the age-related decline in creatinine clearance was accelerated. These results seem to confirm that occupational exposure to hydrocarbons is a non-specific factor that may promote a deterioration of renal function.

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Laminin is a ubiquitous component of basement membranes that seems to play an important structural and functional part within basement membranes. Indeed, this glycoprotein possesses binding domains for heparan sulphate proteoglycan and nidogen and cell binding properties involved in the anchorage of epithelial and endothelial cells to the glomerular basement membrane. Polyanionic charges of heparan sulphate proteoglycan are probably implicated in the ionic control of filtration through the glomerular basement membrane and nidogen mediates the binding of laminin to collagen IV, another major component of basement membranes.[1] As the normal renal filtration process is highly dependent on an intact glomerular basement membrane it is not surprising that the serum laminin concentration may increase[2] and that antilaminin antibodies may appear in patients diagnosed with renal disease.[3]

As organic solvents are suspected of playing a part in chronic nephropathies, Viau *et al*[4] searched for antilaminin antibodies in refinery workers exposed to hydrocarbons and found a slightly increased prevalence of high titres in exposed workers. Furthermore, Mutti *et al*[5] have recently described an increased serum laminin concentration in workers exposed to perchloroethylene. In both studies however, workers diagnosed with a renal or systemic disease were excluded. This might cause a selection bias as the workers with a history of kidney disease might constitute the group most at risk. Indeed, if renal disease is worsened by occupational exposure to hydrocarbon, the renal disease will progress more rapidly and it will be discovered earlier. Therefore, these workers will be excluded although hydrocarbons may have played a part in the progression of their renal disease. As a selection process of this kind is thought to be an explanation for the paradoxical association between albuminuria and ethnicity found in the cross sectional study of Gerber *et al*,[6] the possibility that studies including only healthy workers have underestimated the real risk of hydrocarbon nephrotoxicity in occupationally exposed workers cannot be excluded.

The purpose of this study was to measure the serum laminin concentration in a population of workers exposed to hydrocarbons, including subjects diagnosed with previous or present renal disease or hypertension or both. The hypothesis was that a disturbance in the glomerular basement membrane is induced by the interaction between hypertension and occupational exposure to hydrocarbons and, therefore, that serum laminin concentration would increase especially in the exposed and hypertensive workers. This hypothesis is based on the results of a previous study with other markers of renal damage.[7 8] We also attempted to confirm these results with a biological indicator of exposure rather than indirect exposure indices such as exposure duration and exposure score. Therefore, a group of toluene exposed printers whose hippuric acid excretion was known was examined. Finally, the relations between serum laminin or heparan sulphate proteoglycan (assessed as total urinary glycosaminoglycan excretion) and other markers of glomerular damage were examined.

Subjects and methods

The study population involved adult male workers who were either invited to a general health examination in the frame of studies assessing occupational risks or had to undergo a periodic or preplacement medical examination. To avoid a recall bias, patients referred to us because of a possible occupational disease were excluded.

A detailed description of the selection rationale, of the products used (mainly toluene alone or in combination), of the characteristics of the subgroups, and of the study protocol has been reported elsewhere.[7 9] Six subgroups were identified—namely, floor layers (heavy hydrocarbon exposure; n = 106), printers (moderate exposure; n = 25), roadmen (slight exposure; n = 40), controls (no exposure at all, n = 48), formerly exposed workers (former exposure only; n = 35), "false" controls (present exposure not suggested by the present job title; n = 10). Fifteen (5·4%) workers did not participate (10 refusals, five because of organisational problems). There was not enough serum for laminin analysis in 20 cases and five workers had no satisfactory urine collection (protocol not observed, too little urine).

A hydrocarbon exposure is defined as an exposure resulting from any of 27 occupations listed in a standardised questionnaire. The exposure indicators are the same as in our previous work.[7 8] Briefly, lifetime exposure duration (LED, years) and lifetime exposure score (LES; arbitrary units, years × intensity factor) were assessed on the basis of the occupational history. Three exposure duration subgroups were used (ED1, no hydrocarbon exposure at all: ED2, between 0·01 and 9·9 years, ED3; $\geqslant$10 years). Work related hydrocarbon induced symptoms (feeling drunk, having a non-specific gastric disturbance, dizziness, nausea, alcohol intolerance, or drowsiness) occurring during the four to eight weeks before the clinical examination were considered as a crude index of recent excessive exposure to hydrocarbons. A symptom was defined as work related if there was a clear work related pattern and when this relation was considered as probable. Doubtful or possible relations were therefore classified as non-work related. Workers complaining of at least one such work related symptom were compared with workers without any such symptom. Workers complaining of symptoms of this kind that could not be considered as work related constituted a third subgroup.

In the printer group, the exposure could also be estimated on the basis of hippuric acid (HA) concentrations in urine. As the individual HA concentration had generally been measured every three months since 1978, individual yearly means of HA excretion could be calculated. Their sum for each worker gives the individual cumulative HA excretion. Details about these surveys and the analytical methods can be found elsewhere.[10] The examined printers represent the whole present workforce of a printing house where lead was never used.

Exposure to lead, mercury, or cadmium was looked for with a check list. If possible, an intensity score was also assessed blindly by an industrial hygienist.[7]

Serum laminin (U/ml) was assayed by measuring its pepsin resistant fragment (laminin P1; Behringwerke, Marburg, Germany). Creatinine in blood and urine was assayed by the Jaffé method (Boehringer Diagnostica, Mannheim, Germany) and the endogeneous creatinine clearance was measured over some hours (ml/min/1·73 m^2; median sampling time 187 minutes). Albumin excretion rate (U-alb; μg/min) was assayed by radioimmunoassay (Pharmacia, Uppsala, Sweden). The fractional albumin clearance was calculated as (urinary albumin/serum albumin) × (plasma creatinine/urinary creatinine). N-acetyl- β-D-glucosaminidase activity (NAG; mU/mmol creatinine), glycosaminoglycan concentration (GAG), erythrocyte counts (number/ml), retinol binding protein (RBP; μg/mmol creatinine), serum β-2-microglobulin (S-β2MG; mg/l), serum albumin (g/l), and protein:creatinine ratio in the first and second morning urine (PCR-U1 and PCR-U2) were assessed as described previously.[7 8 11] A PCR-U1>108 or a PCR-U2 > 127 indicate abnormal proteinuria. The GAG excretion was expressed as a concentration (mg/l) standardised for a diuresis of 1 ml/min.[8]

Several confounders were considered because serum laminum may be affected by liver dis-

eases,[12 13] scleroderma,[14] cancer,[15] diabetes,[16] and perhaps age or glomerular filtration rate.[2] All workers who had a clinical history with past or present disease capable of affecting the kidney (systemic diseases such as hypertension or diabetes included) or the urinary tract, or who had regularly taken drugs for at least one month before the clinical examination constituted the "renal" disease group. Regular drug consumption was taken into consideration because it can affect sensitive markers of renal disease. Most workers in this group (52; 44·1%) were hypertensive (systolic blood pressure $\geqslant 160$ or diastolic pressure $\geqslant 95$ mm Hg). There were too few workers with renal parenchymal disease to constitute a more specific group.

As Gosling and Beevers[17] showed that U-alb can already increase above blood pressure values $\geqslant 140$ or $\geqslant 90$ mm Hg, we also defined subgroups according to these cut off values. A "higher" blood pressure (HBP) was then defined as any value $\geqslant 140$ or $\geqslant 90$ mmHg, a "lower" blood pressure (LBP) as values <140 and <90 mm Hg. Mean blood pressure is the sum of diastolic blood pressure and one third of the difference between systolic and diastolic blood pressure.

The digestive disease group included all workers with a clinical history of digestive, biliary, pancreatic, or hepatic disease, or regular drug consumption as defined earlier. The liver disease group included only workers with a history of hepatic disease. Most patients (11; 58%) had a clinical history of hepatitis. Further confounding factors were creatinine clearance, age, alcohol consumption (in drinks/day[18]), and exposure to heavy metals. No worker suffered from scleroderma or primary biliary cirrhosis.

Logistic regression analysis, variance analyses, and multiple regression (backward method) were used to compare groups or to study a variable.[19 20] If necessary, non-parametric tests or logarithmic transformations were used. For exposure score or exposure duration a normalisation or a better residual distribution could only be achieved by taking the square root.

Results

Age, body weight, body height, smoking (pack-years), LED, LES, S-β2MG, S-creatinine, creatinine clearance, PCR-U2, U-alb, GAG, and the prevalences of workers with symptoms of excessive exposure, daily alcohol consumption, liver or "renal" disease, and hypertension were compared between the group with missing values for one or more of the most important biological measures (serum laminin, U-alb, GAG) and the group without missing values. Most missing values (n = 20; 80%) was due to a missing serum laminin determination (too little serum). These comparisons did not show any trend with respect to exposure, renal function, or possible confounders that could have biased the results. Medians (10th-90th percentiles) were 37 years (21–57) for age, 8·9 years (0–30·2) for LED, 11·5 arbitrary units (0–42·8) for LES, 1·87 U/ml (1·51–2·37) for S-lam, 1·70 mg/l (1·30–2·10) for S-β2MG, 91·0 μmol/l (79·0–103·0) for serum creatinine, 110·4 ml/min/1·73 m^2 (78·3–137·4) for creatinine clearance; 103·3 (77·8–171·1) for PCR-U2; 5·08 μg/min (2·40–20·28) for U-alb, and 45·6 (34·5–62·5) for GAG (concentration standardised for a diuresis of 1 ml/min), in the group without missing values.

Concentrations of serum laminin correlate with age ($r = 0{\cdot}17$, $p \leqslant 0{\cdot}001$) and systolic and diastolic blood pressure ($r \geqslant 0{\cdot}14$, $p \leqslant 0{\cdot}005$). Correlations with serum creatinine, S-β2MG, or creatinine clearance were at best of borderline significance ($p \geqslant 0{\cdot}07$). The serum laminin concentrations were not raised in the digestive disease group ($p = 0{\cdot}29$) and did not show any pattern related to smoking ($p = 0{\cdot}22$) or alcohol consumption ($p = 0{\cdot}32$), which was less than about 40 g ethanol/day in 240 (90·9%) workers. A slight but not significant trend towards higher values was found in the liver disease group ($p = 0{\cdot}39$) whereas the increase was clear in the "renal" disease group ($p = 0{\cdot}01$) and still more in the hypertensive group or the group with higher blood pressure ($p \leqslant 0{\cdot}0001$) although there were few patients with severe hypertension (90th percentile : 158 and 98 mm Hg, highest values 200/100 and 180/120 for systolic and diastolic blood pressure respectively). Belonging to the liver or "renal" disease group and blood pressure, age, and creatinine clearance were therefore considered as possible determinants in further analyses.

In a first step, the influence of LED or LES, and blood pressure or "renal" disease on serum laminin concentration was examined before taking possible confounders into account. Table 1 shows that the blood pressure increased serum laminin concentration more in the exposed than in the non-exposed workers. The same trend was found in the worker group with long duration of exposure ($\geqslant 10$ years). Table 1 is designed to allow a direct comparison with our previous results and shows that serum laminin behaved similarly to U-alb or NAG.[7] The other possible combinations (LES quartiles with the factor "renal" disease, or ED1-3 with LBP or HBP groups) showed a similar trend (details not given). In the multiple regression analysis the interaction term between mean blood pressure and exposure index (LED or LES) was always statistically significant and furthermore, the coefficients remained fairly stable after the introduction of the confounders (table 2). As discussed later, the negative regression coefficient is probably due to a

Table 1 Occupational hydrocarbon exposure, hypertension, and serum laminin concentration

	Laminin concentration					*Laminin concentration*		
	No of workers	*Median*	*90th percentile*	*p Value*		*No of workers*	*Median*	*90th percentile*	*P Value*
LBP group					*No "renal" disease*				
ESQ1	50	1·87	2·47		ED1	27	1·89	2·83	
ESQ2	48	1·79	2·08		ED2	57	1·79	2·05	
ESQ3	40	1·81	2·26		ED3	46	1·85	2·30	
ESQ4	40	1·81	2·37						
HBP group					*With "renal" disease*				
ESQ1	10	1·96	2·34	0·54	ED1	8	2·18	—	0·22
ESQ2	15	2·13	2·47	0·006	ED2	27	1·78	2·33	0·33
ESQ3	13	2·04	2·72	0·003	ED3	70	1·95	2·40	0·07
ESQ4	24	1·99	2·56	0·007					

See methods section for definitions of LBP, HBP, "renal" disease group, and ED1–3; ESQ1–4 = exposure score quartiles (from 1 = lowest to 4 = highest); p by Mann-Whitney U test for the difference between LBP and HBP workers in each ESQ or between "renal" disease groups in each ED category.

selection effect. Concentrations of serum laminin were not raised in the workers complaining of symptoms of excessive recent exposure. The same calculations were done with the present job as an index of present exposure but some subgroups were too small to allow a meaningful statistical analysis.

We also assessed the two basal membrane components, serum laminin and GAG, as possible predictors of glomerular damage. The markers of glomerular damage used were total proteinuria, erythrocyturia, and U-alb. Neither serum laminin nor GAG were clearly associated with PCR values and neither was a significant predictor of abnormal proteinuria (PCR-U1 > 108 or PCR-U2 > 127). GAG but not serum laminin was weakly associated with an increased erythrocyturia (> 600 erythrocytes/ml) ($p = 0·05$). In the multiple regression analysis with GAG excretion and serum laminin concentration, GAG excretion, but not serum laminin concentration, correlated with U-alb (determination coefficient 0·13). After considering RBP concentration (as an indicator of proximal tubule dysfunction) and NAG activity (as an indicator of tubular damage), the determination coefficient increased (0·22). This effect was independent of blood pressure, which still increased the determination coefficient (0·27) without removing the three other variables. In particular, GAG remained a good predictor of albuminuria (partial regression coefficient: 0·009, $p < 0·0001$) whereas the serum laminin effect was non-significant in all regressions. The same results were obtained with the fractional albumin clearance, which is thought to be a more reliable indicator of the properties of the glomerular filter than the albumin excretion rate.

It could be argued that the interaction between blood pressure and exposure for serum laminin concentration is questionable because the exposure indicators are based on the occupational history only. Therefore, the regression analyses were done again after substitution of cumulative HA excretion in the LED or the LES. Simultaneously, the exposure effect on the variables (U-alb, NAG, and to a lesser extent PCR or erythrocyturia) that proved interesting in our previous work[7] were re-examined with this exposure index. Only the printers (n = 25) and the controls (n = 48) were included in these analyses. Care was taken to exclude six printers who had had an important solvent exposure other than that due to work in the plant where

Table 2 Serum laminin

Exposure index		*Other independent variables*				*Adjusted r²*
		MBP	*Age*	*Interaction*	*"Renal" disease*	
LED	−0·22	R	0·001	0·11	R	
(n = 240)	(0·0001)		(0·01)	(0·0001)		0·09
LES	−0·18	R	0·001	0·09	R	
(n = 240)	(0·0003)		(0·005)	(0·0004)		0·09
cumulative HA	−0·75	R	R	0·37	R	
(n = 62)	(0·03)			(0·03)		0·06

Dependent variable = serum laminin. Each multiple regression analysis considers another exposure indicator but the same other independent variables. The values indicated are partial regression coefficients (B) and levels of significance (in parentheses); R = removed from the backward regression analysis (non-significant); r^2 = adjusted determination coefficient; MBP = mean blood pressure. Interaction = interaction between MBP and LED, LES, or cumulative HA, respectively; for renal disease; 1 = no, 2 = yes.

Table 3 Multiple regression analysis with albumin excretion rate as dependent variable

Set of independent variables							
GAG excretion	*Serum laminin concentration*	*Cumulative HA*	*MBP*	*Interaction*	*Renal disease*	*RBP concentration*	r^2
1·21 (0·008)	R	NC	NC	NC	NC	NC	0·10
NC	NC	−4·23 (0·01)	R	2·11 (0·01)	R	NC	0·07
1·21 (0·008)	R	−3·75 (0·03)	R	1·88 (0·03)	R	NC	0·17
1·20 (0·009)	R	−3·73 (0·03)	R	1·87 (0·03)	R	R	0·16

Each equation was run with a different set of independent variables; the partial regression coefficient (B) and the level of significance (in parentheses) are indicated; NC = none considered in this run; R = removed from the backward regression analysis (non-significant); MBP = mean blood pressure; r^2 = adjusted coefficient of determination; n = 66 (after exclusion of 6 workers; see text).

the biological monitoring was done. The interaction between blood pressure and exposure increased significantly the albumin excretion rate (table 3), the fractional albumin clearance (partial regression coefficient of the interaction: 2·18, p = 0·01), the serum laminin (table 2), and the NAG activity (partial regression coefficient: 1·27, p = 0·003). This interaction effect was also associated with an abnormal PCR-U1 and PCR-U2 (r = 0·25 and 0·20, p = 0·01 and 0·03 respectively; logistic regression analysis). Only the erythrocyturia was not dependent on this interaction.

As the decline in creatinine clearance is age related, the interaction age-cumulative HA excretion was used to examine the interaction effect on this variable. Again the interaction was significant (partial regression coefficient = − 0·60, p = 0·01). The results were similar if S-β2MG was used instead of creatinine clearance.

This interaction effect cannot be easily explained by confounders as the known confounders were considered in all equations and as the inclusion of the six excluded printers (see earlier) did not change the results. Moreover, these results cannot be explained by a high correlation between LED or LES and cumulative HA excretion because this correlation is weak (r = 0·16, p = 0·25). As in the multiple regression analysis with age, body mass index, regular drug taking, cumulative HA excretion, and the factor "renal" disease, only age and body mass index were associated with mean blood pressure, an association between mean blood pressure and cumulative HA excretion cannot be viewed as a confounder. Finally, as heavy metal exposure was more frequent in the control group than in the printers' group (50·0% *v* 8·1%) it cannot be made responsible for the effects found.

It should be emphasised that the printers' exposure to toulene was never very high between 1978 and 1987. The median of the individual mean yearly HA excretions for this 10 year period ranged from a maximum of 1·5 in 1984 to a minimum of 0·5 mmol HA/mmol creatinine in 1987. The yearly median excretion diminished (range of medians: 1·1–1·5 between 1978 and 1984, 0·5–1·0 between 1985 and 1987). The highest individual yearly mean was 3·3 mmol HA/mmol creatinine and was registered in 1984 (present biological exposure limit: 1·58 mmol HA mmol/creatinine). The median cumulative HA excretion was 10·1 mmol HA/mmol creatinine (range: 0·3–15·3). Enough workers remained in the plant between 1978 and 1987 to allow comparison of their yearly HA excretion with the yearly HA excretion of those who left the printing house. A cross sectional comparison of the yearly HA excretion of the two groups did not show differences between them for any year (0·14 $\leqslant$ p $\leqslant$ 0·99) if the comparison was made year by year. An analysis of the cumulative HA excretion over the whole employment time, however, showed that the printers' group was not homogeneous. There was an obvious trend towards a lower exposure intensity in the workers who remained in the plant for a long time (p = 0·0002).

Discussion

The most interesting result from this study was the finding of an interaction between hypertension and cumulated HA excretion in a population of printers exposed to toluene whose urinary HA excretion was regularly monitored. This interaction was significantly associated with an abnormal proteinuria, an increased serum laminin concentration, albumin excretion rate, and NAG activity. It cannot be explained by the confounders studied. Furthermore, exposure to hydrocarbons seemed to accelerate the age dependent decline in creatinine clearance. These results agree well with the conclusions drawn for several markers of kidney damage in a hydrocarbon exposed population in which anamnestic exposure indices (LED, LES) were used instead of biological monitoring.[7 8]

The second interesting result was the relation between U-alb and the two markers of glomerular basal membrane disturbances, serum laminin concentration and GAG excretion. Although both serum laminin concentration and GAG excretion are thought to increase in glomerular damage[2 21] GAG, but not serum laminin seems to be a predictor of U-alb, probably because its negative charges repel the albumin molecule and limit its filtration.[22] The hydrocarbon metabolites and hypertension could induce a metabolic disturbance at the level of glomerular basal membranes causing a loss of polyanionic charges and an increased escape of albumin. One possible explanation of these relations might be that hypertensive workers produce more nephrotoxic metabolites in the form of thioethers than normotensive workers. This hypothesis is based, however, only on animal experiments[23 24] and has to be verified in humans.

The third interesting point is the influence of hydrocarbon exposure and hypertension on the serum laminin concentration. In this regard, the serum laminin concentration showed the same pattern as that already described for several other markers of glomerular and tubular damage with LED and LES.[7 8] The lack of effect of alcohol and hepatitis is not surprising because the study population included few heavy drinkers and because most of the hepatitis cases were probably of type A. In the same study population, alcohol consumption increased GGT and ALT activities ($p \leqslant 0{\cdot}0001$ and $p = 0{\cdot}09$ respectively, details not shown) so that the lack of effect of alcohol consumption on serum laminin concentration cannot be explained by inaccurate answers to the question about alcohol consumption. With respect to the exposure indices the negative partial regression coefficient may be explained by a selection effect due to the cross sectional study design. Hypertensive workers have a higher risk of cardiovascular complications and, therefore, are more likely to leave the plant because of disease. Furthermore, as shown by the relation between cumulative HA excretion and exposure duration in the printer group, it seems that the most heavily exposed workers, who should have the greatest risk of kidney damage, tended to leave the plant earlier. Selection because of disease and because of heavy exposure should therefore cause the progressive formation of a subgroup of normotensive and healthy workers with a fairly long exposure duration but a rather low exposure intensity. Consequently, as both the cumulative indices and the number of years during which the selection effect works increase with time, the partial regression coefficient becomes negative after taking into account the interaction between hypertension and exposure.

The fact that the lack of homogeneity of the study population is not apparent at first sight is not surprising if the limitations of these exposure indices in the frame of a cross sectional design are considered. The LED only takes the exposure duration into account and neglects the individual differences of the exposure intensity. The LES and the cumulative HA excretion consider both exposure duration and intensity but are unable to distinguish between some years of heavy exposure and many years of low exposure. Only the examination of the exposure of all workers having ever being hired during a period of several years can disclose these exposure differences. We are aware that the lack of medical data does prevent us from proving this explanation but it should be borne in mind that the results of the different kidney function tests are consistent[7 8 11] and that all important known confounders have been considered for each test without changing the results. Moreover, these opposite processes might also partly explain the fact that cross sectional studies about the nephrotoxicity of organic solvents have often produced conflicting results.

It could be argued that the study results rely on sensitive biochemical markers that are not relevant for prevention. This objection does not apply, however, to abnormal proteinuria or to increased albuminuria. Indeed, both are associated with the progression of renal disease and an increased prevalence of cardiovascular disease.[25-28] Furthermore, occupational exposure to hydrocarbons is also associated with glomerulonephritis and chronic renal failure.[29 30] Owing to the occupational, medical, social, and financial consequences of chronic renal disease the practical importance of good control of occupational hydrocarbon exposure is obvious.

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